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Design, synthesis, and pharmacological evaluation of *N*-(4-mono and 4,5-disubstituted thiazol-2-yl)-2-aryl-3-(tetrahydro-2*H*-pyran-4-yl) propanamides as glucokinase activators

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ABSTRACT

A series of *N*-thiazole substituted arylacetamides were designed on the basis of metabolic mechanism of the aminothiazole fragment as glucokinase (GK) activators for the treatment of type 2 diabetes. Instead of introducing a substituent to block the metabolic sensitive C-5 position on the thiazole core directly, a wide variety of C-4 or both C-4 and C-5 substitutions were explored. Compound *R*-**9k** bearing an *iso*-propyl group as the C-4 substituent was found possessing the highest GK activation potency with an EC_{50} of 0.026 μM. This compound significantly increased both glucose uptake and glycogen synthesis in rat primary cultured hepatocytes. Moreover, single oral administration of compound *R*-**9k** exerted significant reduction of blood glucose levels in both ICR and *ob*/*ob* mice. These promising results indicated that compound *R*-**9k** is a potent orally active GK activator, and is warranted for further investigation as a new anti-diabetic treatment.

1. Introduction

Type 2 diabetes mellitus (T2D) is a progressive heterogeneous disease affecting over 250 million patients worldwide in 2007.[1] It is characterized by insulin resistance, excessive hepatic glucose production, and reduced glucose-triggered insulin secretion from the pancreatic β-cells.[2–7] Although a number of T2D-pathogenesis-targeting drugs have been marketed as anti-diabetic therapies, no single current available drug is capable of achieving sustained blood glucose control.[8,9] Glucokinase (GK), one of the four hexokinase isozymes,[10] is a glucose-phosphorylating enzyme, which is highly expressed both in the pancreatic β-cells as a glucose-sensor maintaining plasma glucose homeostasis[11–13] and in the liver as a rate-determining factor regulating glucose metabolism.[14–19] Therefore, GK has become an emerging anti-diabetic target and hundreds of small molecule compounds have been designed in the past few years with the hypothesis that compounds targeting this enzyme would activate the allosteric pocket of this protein and achieve anti-hyperglycemic effects by enhancing glucose usage in the liver and by potentiating insulin secretion from pancreatic β-cells.[20–34] However, compounds with significant GK enzyme potency less than 1 μM were very limited, and only a few have approached to the clinic.[27–32]

Among the reported small molecule GK activators (GKAs), benzamides, and phenylacetamides are the major structural scaffolds. The former scaffold is represented by the phase II clinical compound **1** (**GKA50**)[35–37] which was developed by AstraZeneca possessing good in vitro enzyme potency (EC_{50} = 30 nM) and appreciable PK properties both in rats (*F* = 90%) and dogs (*F* = 100%), whereas the latter chemotype is exemplified by compound **3** (**PSN-GK1**)[26,33,38] which is a back-up of compound **2** (failed in clinic) and currently in phase I clinical trial (Fig. 1). Both compounds **2** and **3**, structurally analogous to the initial high-throughput-screening (HTS) hit **4** (Ro-28-1675)[20,39] of Roche, have high GK activation potencies with EC_{50} values of 570 nM and 130 nM, respectively, while compound **2** showed extremely toxic in acute toxicology studies in rats.[33] The toxicity of compound **2** was postulated to be the oxidative metabolism potential of the aminothiazole component leading to a toxic thiourea metabolite in vivo, therefore, compound **3** was designed with a fluoro atom to block the oxidation-sensitive position (C-5) of the thiazole core (Fig. 2).[33] This strategy has been proven effective and compound **3** showed improved pharmacokinetics and better safety profile.[26,33,38] Although a number of arylacetamides with other substitution patterns on the thiazole fragment were also claimed in Fyfe and his colleagues' publication and patent at OSI Pharmaceuticals,[33,38] only limited substituents at the 4- or 5-position (Me, H, COOEt, COOH, etc.) have been reported with biological data.

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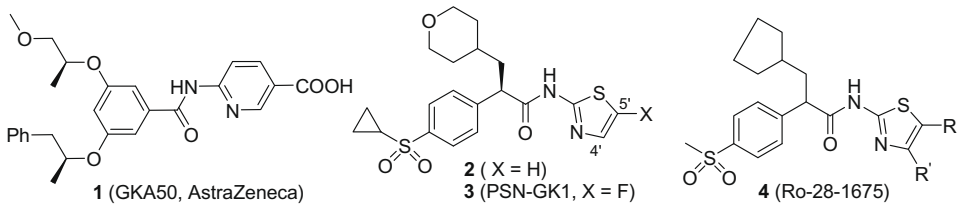


Figure 1. Representative GK activators.

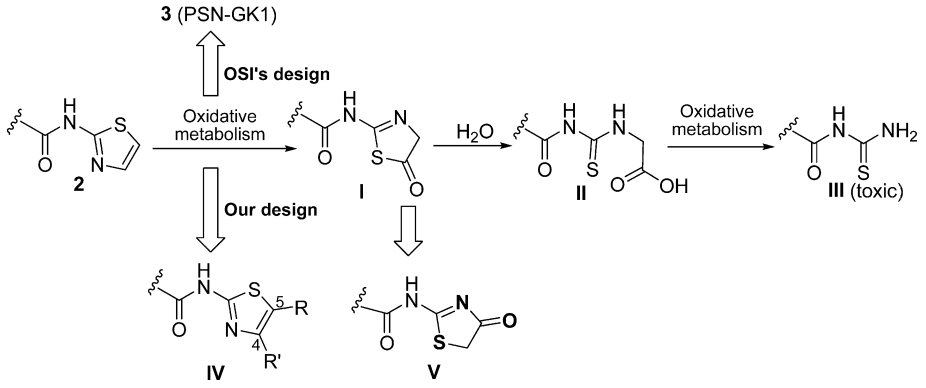


Figure 2. Drug design strategy based on metabolic mechanism.

Since it has been confirmed[33] that an aminothiazole compound like **2** would readily undergo oxidation at the 5-position of the thiazole ring in vivo to yield a thiazol-5-one (**I**), which is then hydrolyzed to thioureidoacetic acid (**II**) through a ring-opening path, followed by a second oxidation process leading to the actual toxin—thiourea species **III** (Fig. 2). Therefore, a direct blockade at 5-position of the aminothiazole core would possibly terminate the metabolic process and inhibit the production of the toxic thiourea **III**. In our metabolic mechanism-based drug design program, we envisioned that an appropriate neighboring substituent at the C-4, instead of direct blocking C-5 position of the thiazole fragment, may exert indirect influence to the metabolic stability of C-5. Meanwhile, C-4 and C-5 double substitutions may also create compounds with better pharmacological profile. Moreover, structural modification directly on the metabolites (**I**–**III**) may also lead to novel compounds retaining GK activation potency but lacking the unwanted metabolic concerns. Therefore, a series of metabolism-based arylacetamides (represented by Markush structures **IV** and **V**) (Fig. 2) were designed as analogues of compounds **2–4** with variant substituents at C-4 or both C-4 and C-5 (other than F-) positions on the thiazole core, with the aim to identify additional lead compounds possessing more efficacy and better pharmacokinetic properties. Herein, we described our synthesis of these compounds and their pharmacological evaluation in vitro and in vivo.

2. Results

2.1. Chemistry

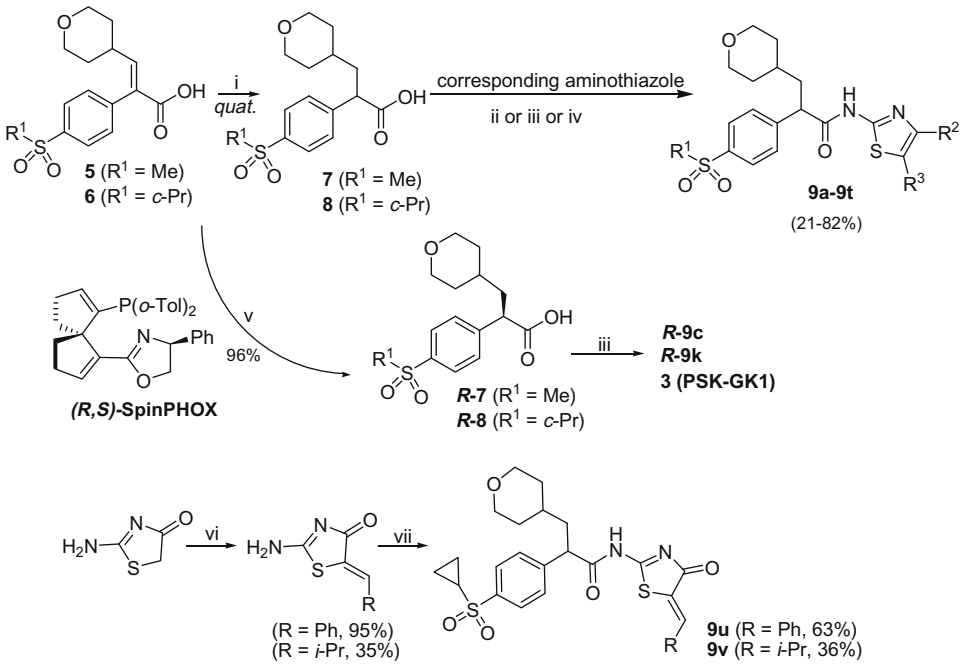
To quickly identify promising compounds for in vivo studies, most of the α-(tetrahydro-2*H*-pryan-4-yl)methyl-α-arylacetamides were synthesized as racemates for preliminary evaluation since the GK activation potencies of the racemate and *R*-enantiomer of the initial lead **4** or clinical compound **3** do not differentiate from each other significantly in vitro, although only *R*-enantiomers were active in vivo.[20,33] Condensation of 4-methylsulfonyl-(**7**)[33] or 4-cyclopropylsulfonyl-(**8**)[33] substituted phenylacetic acid with corresponding aminothiazole was conducted following a standard literature procedure.[33,38] For the most potent compounds, their *R*-configurated enantiomers were prepared by using *R*-configurated acid *R*-**7** or *R*-**8**, which were prepared in 95% ee, respectively, by enantioselective hydrogenation[33,38] of acrylic acids **5** and **6** with Ir(I) complex of SpinPHOX (spiro[4,4]-1,6-nonadiene-based phosphine-oxazoline) following a procedure[40] we reported recently.

4-Mono, or 4,5-double substituted aminothiazoles which were used for the preparation of arylacetamides **9a**–**s** were directly purchased from commercially available sources, and subsequent condensation with acid **7** or **8** occurred smoothly leading to products **9a**–**s** in 21–82% yield. The low yields in several cases were due to the sluggish reaction process and low substrate conversion. Compounds **9t–v** were prepared as bioisosteres of the first generation metabolite (**I**) of **2** by shifting the carbonyl group or its equivalents from C-5 to C-4 of the aminothiazolone component. The condensation precursors (*Z*)-4-benzylidene-4,5-dihydrothiazol-2-amine (for **9u**) and (*Z*)-4-(2-methylpropylidene)-4,5-dihydrothiazol-2-amine (for **9v**) were prepared[41] from 2-aminothiazol-4(5*H*)-one with benzaldehyde or isobutyraldehyde in refluxed AcOH in 95% and 35% yield, respectively (Scheme 1).

2.2. Glucokinase enzymatic assays

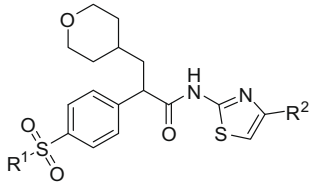
All new synthetic compounds were evaluated in an enzymatic glucokinase (GK) assay using purified recombinant human islet GK. GK activity was measured in a coupled reaction with glucose-6-phosphate dehydrogenase (G6PDH) through monitoring nicotinamide adenine dinucleotide phosphate (NADPH) production by the increase rate of absorbance at 340 nm in a Molecular Device SpectraMax 190 plate reader. The assay protocol is similar to that reported,[20,33,42] and the GK activity of each compound was evaluated in six different concentrations and was reported as EC_{50} value and maximum activation fold (vs control level). Known compounds **9a**,[33] **3** (PSN-GK1)[33,38] and its racemic form *rac*-**3**[38] were also evaluated in our assays for comparison.

To evaluate the effect of a C-4 substituent in the aminothiazole core on the GK activation, a series of racemic methylsulfonyl substituted arylacetamides **9a**–**i** were first assayed (Table 1).



Scheme 1. Reagents and conditions: (i) 10% Pd/C, H_2; (ii) for compound **9a**: TBTU, Et_3N or DIPEA, CH_2Cl_2, rt; (iii) for compounds **9b**–**s**: PyBOP, Et_3N or DIPEA, CH_2Cl_2, rt to reflux; (iv) for compound **9t**: $(COCl)_2$, DMF, collidine, CH_2Cl_2, −20 °C to rt; (v) $[Ir(COD)Cl]_2$, (*R,S*)-SpinPHOX, S/C = 100, H_2, Et_3N, MeOH, 50 °C, 20 h, 30 atm; (vi) RCHO, AcOH, reflux, 24h; (vii) acid **8**, TBTU, DIPEA, CH_2CH_2, 25 °C, 12h.

Table 1
In vitro activation of GK by 4-substituted-thiazole analogs



Compd	R^1	R^2	EC_{50} (μM)	Max act[b]	Compd	R^1	R^2	EC_{50} (μM)	Max act[b]
3(PSN-GK1)	—	—	0.062 (0.13[a])	2.59	**9g**	Me	Ph	0.724	1.30
rac-**3**	—	—	0.089	4.6	**9h**	Me	*p*-MeO-Ph	1.04	1.22
9a	Me	H	1.69 (8.9[a])	2.45	**9i**	Me	*p*-F-Ph	1.18	1.25
9b	Me	Et	1.00	2.17	**9j**	*c*-Pr		0.158	2.40
9c	Me	*i*-Pr	0.310	2.49	*R*-**9c**	Me	*i*-Pr	0.33	2.10
9d	Me	*i*-Bu	0.355	2.05	*R*-**9k**	*c*-Pr	*i*-Pr	0.026	2.55
9e	Me	*t*-Bu	2.93	1.29	**9k**	*c*-Pr	*i*-Pr	0.157	2.97
9f	Me	*c*-Pr	1.35	1.87					

[a] Data from Ref. 33.
[b] Maximum fold activation of GK over control level.

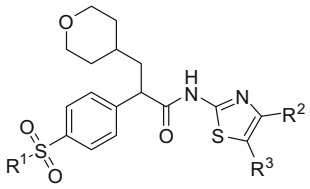
Compared to non-substituted aminothiazole **9a**,[33] all these C-4 substituted compounds displayed comparable or even improved GK potency. Aminothiazoles **9c** and **9d** with moderate size C-4 substituents gave >4-fold increase of potency with EC_{50} values of 0.31 and 0.35 μM, respectively, whereas compounds **9b** and **9f** with smaller or slightly steric substituents led to lower potency, but still slightly more potent than non-substituted aminothiazole **9a**. More steric substituent such as *t*-Bu in **9e** caused a larger decrease in potency. The C-4 aryl substituted aminothiazoles **9g–i** also showed a moderate increase of potency with EC_{50} values of 0.72, 1.04, and 1.18 μM, respectively. A *para*-electron-donating (**9h**) or electron-withdrawing group (**9i**) in the aryl substituent did not impact the potency at all. Since compound **9c** with a C4-isopropyl substituent in aminothiazole core showed the high potency and maximal activation fold in this small series, its *R*-enantiomer *R*-**9c** (95% ee) was evaluated. As expected, *R*-**9c** displayed high potency with an EC_{50} value of 0.33 μM, similar to that of racemic **9c**. Since the cyclopropylsulfonyl substituent in arylacetamide **3** is one of the key elements responsible for the high GK potency,[33] such substitution pattern was also incorporated to compound *R*-**9c** leading to compound *R*-**9k** (95% ee) which showed a significant enhancement (>12-fold) in potency with EC_{50} value of 0.026 μM and maximal activation fold of 2.55. The potency and activation fold of *R*-**9k** are compatible to that of clinical compound **3** which displayed an EC_{50} value of 0.062 μM in our assay. Its racemate (**9k**) showed fivefold lower potency. The isopropenyl analogue **9j** also displayed

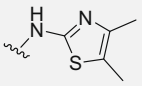
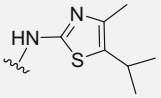
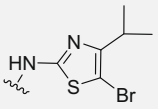
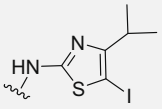
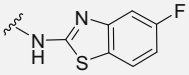
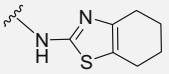
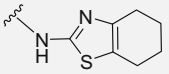
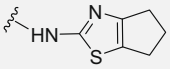
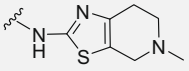
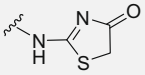
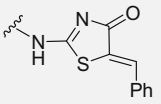
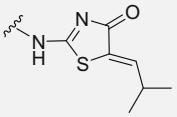
improved potency with an EC_{50} value of 0.158 μM, however, isomerization and decomposition were observed during storage.

Encouraged by the GK activation of C-4 substituted aminothiazoles, a series of C4 and C-5 double substituted aminothiazoles **9l**–**s** were evaluated (Table 2). The simply C-4 and C-5 substituted aminothiazole analogues **9l**–**o** led to significant reduction or loss of potency, except compound **9n** which contains an 4-isopropyl-5-bromo-2-aminothiazole moiety showing an EC_{50} value of 0.32 μM, fivefold more potent than non-substituted aminothiazole **9a**. Aryl or cyclic alkyl fused aminothiazole analogues **9p**–**s** showed a wide difference in GK activation potency. Compound **9p** with an 2-amino-5-fluorobenzo[*d*]thiazole fragment lost GK potency whereas 2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazolo-(**9q**) and 2-amino-5,6-dihydro-4*H*-cyclopenta[*d*]thiazolo-(**9r**) analogues displayed remarkable improvement in potency with EC_{50} values of 0.061 and 0.079 μM, respectively, compatible to that of compound **3**. Insertion of an additional N-atom in the tetrahydrobenzo[*d*]thiazole moiety of **9q** yielded 2-amino-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine **9s** showing significant reduction of potency. 2-Aminothiazol-4(5*H*)-one **9t**, a regioisomer of the first generation metabolite (**I**) of compound **2** showed poor potency. Further derivatives **9u** and **9v** abolished GK potency completely indicating the C-4 carbonyl and C-5 vinyl substituents did not benefit GK interaction. Since compound **9q** is the most potent among the 4,5-double substituted aminothiazole analogues, its *R*-isomer ***R*-9q** was prepared with 94% ee. However, it was found that ***R*-9q** displayed twofold lower potency than its racemate **9q**.

From the results above, 4- and 4,5-double substituted racemic analogues **9k** and **9q** displayed the highest GK potency within each sub-series with EC_{50} values of 0.157 and 0.061 μM, respectively. However, only the *R*-isomer of **9k** (*R*-**9k**) displayed enhanced potency (EC_{50} = 0.026 μM). Therefore, compound *R*-**9k** is the most potent GK activator among our synthetic 4- and 4,5-double substituted aminothiazole analogues, its glucose-lowering potentials were further evaluated in vivo.

Table 2
In vitro activation of GK by 4,5-disubstituted-thiazole analogs



Compd	R^1		EC_{50} (μM)	Activation fold
9l	Me		2.86	1.72
9m	Me		NA[a]	1.13
9n	*c*-Pr		0.32	1.67
9o	*c*-Pr		NA	1.34
9p	Me		NA	1.30
9q	*c*-Pr		0.061	2.27
***R*-9q**	*c*-Pr		0.12	2.55
9r	*c*-Pr		0.079	3.40
9s	*c*-Pr		2.86	2.26
9t	*c*-Pr		4.68	1.65
9u	*c*-Pr		NA	1.24
9v	*c*-Pr		NA	1.17

[a] NA = not active.

2.3. Hepatocyte Assay of R-9k

Glycogen is the primary storage form of glucose, which is critical to glucose homeostasis. Glucokinase exerts a strong control in the glucose influx in hepatocytes, especially in the synthesis of glycogen. Hence, with the most potent compound *R*-**9k** (EC_{50} = 0.026 μM) in the enzymatic assay, we further evaluated its effects on glucose uptake[43,44] and glycogen accumulation[44–46] in rat primary cultured hepatocytes using a general procedure. First, 2-deoxy-D-[^{3}H]-glucose uptake was performed in primary rat hepatocytes in the absence or presence of different concentrations of compound *R*-**9k** in 5.55 mM glucose**.** Relative to control (100%), *R*-**9k** significantly enhanced glucose uptake (180%) with an EC_{50} of 1.33 ± 0.09 μM in hepatocytes (Fig. 3). The glycogen synthesis in primary rat hepatocytes was also enhanced by *R*-**9k** in a time- and dose-dependent manner (Fig. 4a and b). A dose of 20 μM of *R*-**9k** was found sufficient to stimulate glycogen synthesis significantly (p <0.001, 1.83.7 ± 4.5% vs control) after 4 h treatment, and the action reached summit in 9 h. Moreover, the glycogen accumulation in hepatocytes could be enhanced significantly by 1 μM of *R*-**9k** after 6 h incubation (p <0.001, 147.3 ± 7.0% vs control).

2.4. In vivo activity in imprinting control region (ICR) mice

The hypoglycemic effect of *R*-**9k** was evaluated on ICR mice, a non-diabetic animal model.[26] Single oral administration of *R*-**9k** caused rapid and dose-dependent reduction in blood glucose levels in ICR mice (Fig. 5). After 60 min of oral dosing, 3, 10, and 50 mg/kg of *R*-**9k** lowered blood glucose by 12.7%, 33.9%, and 40.7% of vehicle control, respectively. The hypoglycemic effects of *R*-**9k** after single oral dosing could be sustained to 120 min at the dose of 3 mg/kg, and 240 min at the dose of 10 or 50 mg/kg.

2.5. In vivo activity in obese (*ob*/*ob*) mice

The *ob*/*ob* mice is a typical type 2 diabetic animal model which showed hyperglycemia, obesity, and severe insulin resistance.[26,33] Single oral administration of *R*-**9k** significantly decreased the blood

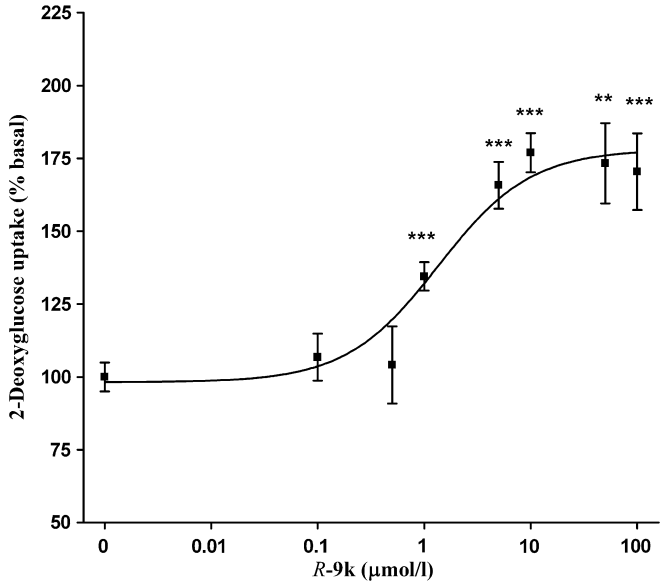


Figure 3. *R*-**9k** stimulates 2-deoxy-D-[^{3}H]-glucose uptake in primary rat hepatocytes in the presence of 5.5 mM glucose. Results are mean ± SEM of three independent experiments. $^{**}p$ <0.01, $^{***}p$ <0.001 versus control.

glucose level of *ob*/*ob* mice. Following a single dose of 20 mg/kg, *R*-**9k** lowered glycemia significantly at time points of 120 and 180 min, whereas 50 mg/kg of *R*-**9k** resulted in a significant decrease at 60, 120, and 180 min after compound administration (Fig. 6).

3. Discussion

Although GK has become a promising target for the development of novel anti-diabetic therapy, and spurred great interest in many pharmacological companies and academia, very limited number of compounds have been found possessing significant GK activation potency, and only a few of them have approached to the clinic. Since compound **2** had been one of the most up-front clinical GK activators and was failed recently in Phase II due to the toxicity of its metabolites, we designed in this report a series of novel arylacetamides with the aim to identify new GK activators without the PK-related safety concerns.

Thiazole analogues **9b**–**k** were prepared to evaluate the effect of a 4-substituent on the GK activation. Most of these compounds displayed improved potency than the non-substituted compound **9a** indicating a lipophilic C-4 pocket may exist in the GK binding sites. *R*-**9k**, bearing an *iso*-propyl group as the 4-substituent of the aminothiazole core was found to be the most potent compound in this series with an EC_{50} value of 26 nM, 2.4-fold more potent than compound **3**. 4,5-Double substituted thiazoles **9l**–**v** were also designed to block the C-5 metabolic position. Most of these compounds showed significantly reduced potency except compounds **9n**, **9q**, and **9r**. 2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazolo- and 2-amino-5,6-dihydro-4*H*-cyclopenta[*d*]thiazolo-analogues **9q** and **9r** displayed remarkably improved potency with EC_{50} values of 0.061 and 0.079 μM, respectively, compatible to that of clinical compound **3**. These results indicated that the 4- and 4,5-positions of the aminothiazole fragment were not just an oxidative metabolic position but also a GK-interaction site.

In the hepatocyte assay, the most potent GK activator, *R*-**9k** significantly enhanced glucose uptake and promoted the glycogen synthesis in primary rat hepatocytes in a time and dose-dependent manner. The good potency in cellular model of compound *R*-**9k** confirmed its endogenous GK activity. In both normal and type 2 diabetic animal models, single oral administration of *R*-**9k** significantly decreased the blood glucose level. These results indicated that compound *R*-**9k** is a potent and orally active GK activator.

To ensure the safety profile of compound *R*-**9k**, its metabolism and plasma stability was investigated using a similar procedure reported by Bertram et al.[33] According to the proposed metabolic mechanism for compound **2** (Fig. 2), compound *R*-**9k** would possibly undergo a similar double-oxidation pathway to yield a same toxic metabolite **III**. Since compound ***R*-9k** produced strong hyperglycemic effect in ICR mice at the dose of 50 mg/kg, the in vivo metabolite analysis of compound *R*-**9k** was performed in Sprague–Dawley (SD) rats with the same dose. Compound *R*-**9k** (50 mg/kg) was orally administered to overnight fasting SD rats. Plasma samples were obtained at 4 h after administration for metabolite analysis by UPLC/Q-TOF MS. Except that dehydrogenation (9.6%; m/z = 461 $[M-H]^+$) and hydroxylation (1.1%; m/z = 495 $[M+2O]^+$) products[56] were detected as the minor metabolites, compound *R*-**9k** was found dominantly remaining in the plasma (89.2%; m/z = 463 $[M+H]^+$), and no trace of metabolite **III** (m/z = 397 $[M+H]^+$) was detected. This result indicated that a C-4 substituent did exert indirect impact on the metabolic stability of thiazole **2**, similar to the direct blockade effect of a C-5 substituent.

4. Conclusion

In summary, a series of *N*-thiazole arylacetamides were designed on the basis of metabolic mechanism of the aminothiazole

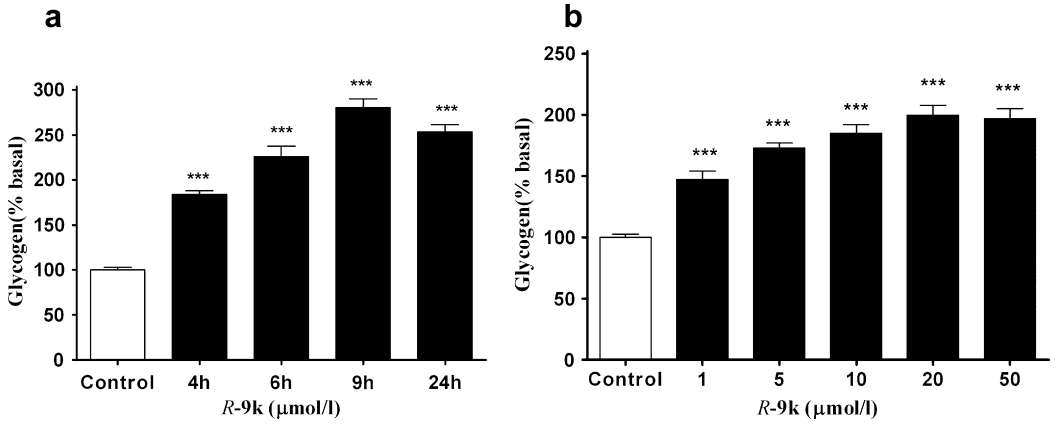


Figure 4. *R*-**9k** increases glycogen accumulation in a time- and dose-dependent manner in rat primary cultured hepatocytes. (a) Time course of glycogen accumulation in the presence of 20 μM *R*-**9k**. (b) Dose–response of *R*-**9k** at the indicated concentrations after 6 h incubation. Results were mean ± SEM of three independent experiments. $^{***}p$ <0.001 versus control.

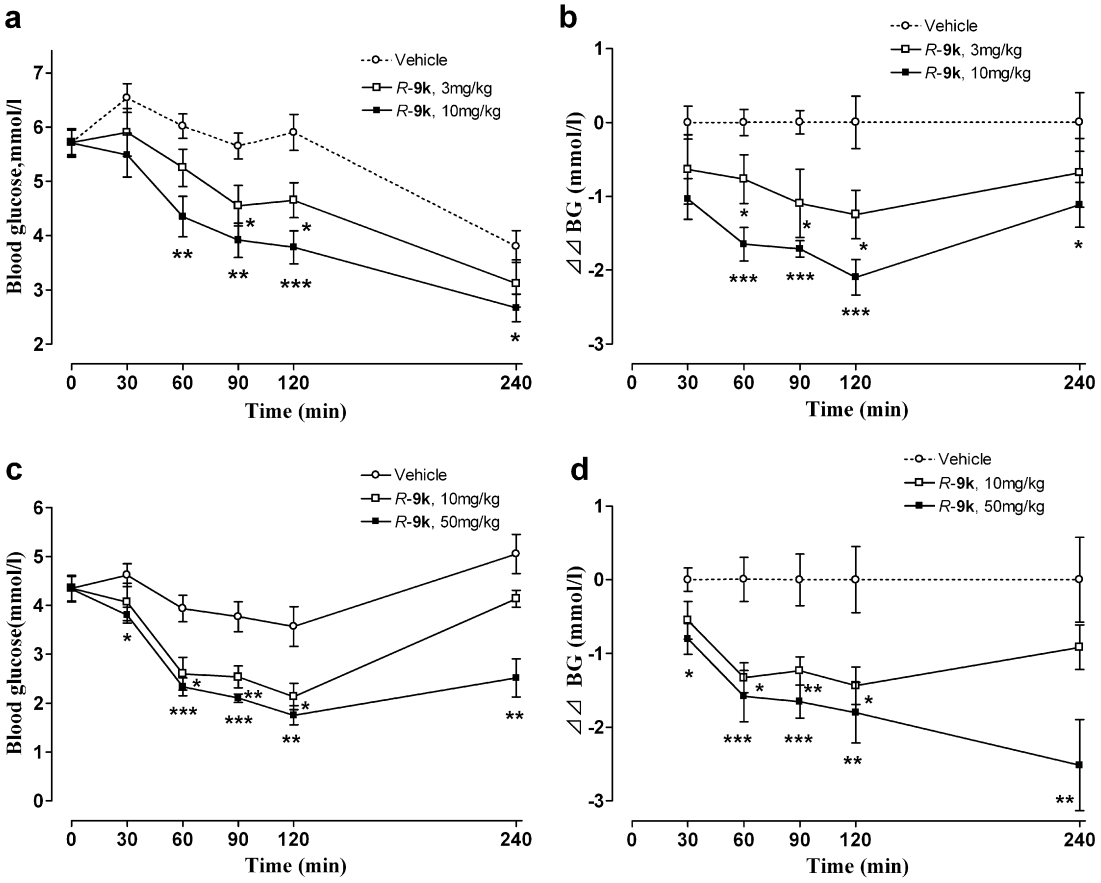


Figure 5. Effects of *R*-**9k** on blood glucose level in ICR mice. (a, c) Blood glucose profiles. (b, d) Delta delta blood glucose ($\Delta\Delta$BG) profiles. $\Delta\Delta$BG reflects the difference between the change in blood glucose concentrations from 0 min within each experimental group (ΔBG) and the change from time 0 min in the vehicle-treated arm. Data were shown as mean ± SEM (n = 6). $^{*}p$ <0.5, $^{**}p$ <0.01, $^{***}p$ <0.001 versus vehicle control.

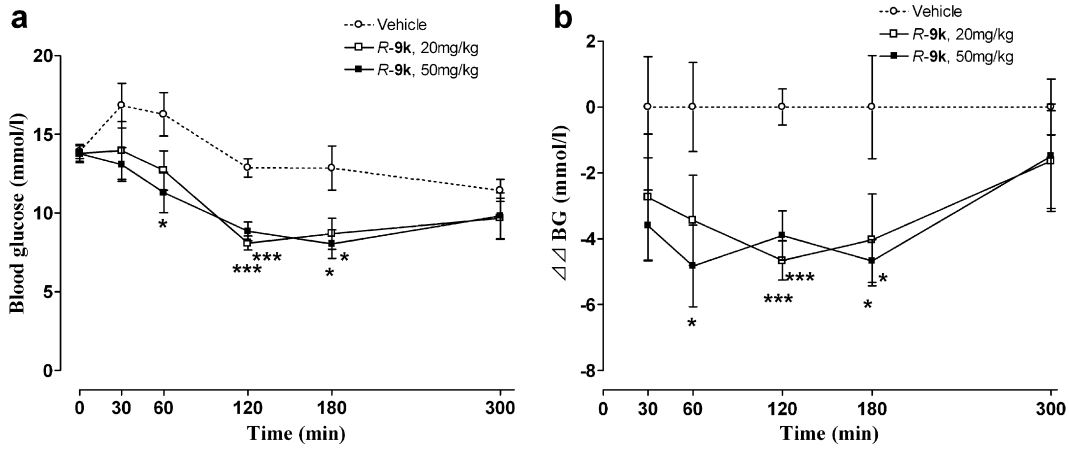


Figure 6. Effects of *R*-**9k** on blood glucose level in *ob*/*ob* mice. (a) Blood glucose profile. (b) Delta delta blood glucose ($\Delta\Delta$BG) profile. Data were shown as mean ± SEM (n = 8). $^{*}p$ <0.5, $^{**}p$ <0.01, $^{***}p$ <0.001 versus vehicle control.

fragment. A wide variety of substituents were introduced to the C-4 and C-4,5 positions of the thiazole fragment. Compound *R*-**9k** containing an *iso*-propyl group as the C-4 substituent was found possessing the highest GK activation potency with an EC_{50} of 0.026 μM. This compound significantly increased glucose uptake and glycogen synthesis in rat primary cultured hepatocytes. Moreover, single oral administration of compound *R*-**9k** produced significant reduction of blood glucose levels in both ICR and *ob*/*ob* mice. These promising results indicated that compound *R*-**9k** is a potent and orally active GK activator, which should be worthy for a further investigation as a new anti-diabetic treatment.

5. Experimental section

5.1. Chemistry

^{1}H NMR spectral data were recorded in $CDCl_3$ on Varian Mercury 300 NMR spectrometer and ^{13}C NMR data were recorded in

$CDCl_3$ on Varian Mercury 400 NMR spectrometer. Low-resolution mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded at an ionizing voltage of 70 eV on a Finnigan/MAT95 spectrometer. Elemental analyses were performed on a CE 1106 elemental analyzer. Optical rotations were determined with a JASCO DCP-1000 digital polarimeter and were the average of three measurements. Column chromatography was carried out on silica gel (200–300 mesh). All reactions were monitored using thin-layer chromatography (TLC) on silica gel plates. Chiral HPLC was conducted on chiralcel OD-H column (hexane/isopropanol, flow rate = 0.7 mL/min, UV detection at λ = 230 nm). Compounds **3**,[33] **7**,[33] **8**,[33] and **9a**[33] were known compounds.

*General procedure for the synthesis of **9a–v** by condensation of acid **7** or **8** with an appropriate aminothiazole:* Method A. To a solution of Arylpropanoic acid **7** or **8** (0.15 mmol), an appropriate aminothiazole (0.225 mmol) and benzotriazol-1-yl-oxy tripyrrolidinophosphonium hexafluorophosphate (PyBOP) (0.14 g, 0.3 mmol) in CH_2Cl_2 (5 mL) pre-cooled in ice-bath, was added dropwise a solution of Et_3N or di(isopropyl)ethylamine (DIPEA) (0.12 mL, 0.75 mmol) and then the mixture was heated to reflux overnight. The solvent was evaporated, and the residue was purified by chromatography (SiO_2, petroleum ether/EtOAc = 2:1 to 1:1) to yield corresponding amides as white or yellow foam. Method B. A mixture of anhydrous CH_2Cl_2 (4 mL) and DMF (17 μL) was cooled to −20 °C, $(COCl)_2$ (22 μL, 0.225 mmol) was added slowly. The mixture was stirred for 45 min, and arylpropionic acid **8** (0.15 mmol) was added. The reaction was continued for 1 h at −20 °C, and collidine (82 mg, 89 μL, 0.675 mmol) was added slowly. Stirring was continued for 15 min, and then an appropriate aminothiazole (0.225 mmol) was added at −20 °C. The resulting suspension was warmed and kept at −15 °C for 2 h, and then at rt overnight. The reaction mixture was evaporated to dryness, treated with EtOAc (10 mL) and 0.1 M HCl (5 mL). The aqueous layer was extracted with EtOAc (2 × 10 mL). The combined extracts were washed consecutively with satd $NaHCO_3$ and brine, and dried over anhydrous Na_2SO_4. After filtration and evaporation, the residue was purified by chromatography ($CHCl_3$/MeOH = 50:1 to 30:1) to give corresponding amides as white or yellow solid. Method C. To a solution of arylpropanoic acid **8** (85 mg, 0.25 mmol) and an appropriate aminothiazole (0.25 mmol) in CH_2Cl_2 (5 mL) cooled in ice-bath was added *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate (TBTU) (0.5 mmol) and Et_3N or DIPEA (1.25 mmol). The reaction mixture was stirred at rt overnight and worked-up as described in Method B.

5.1.1. *N*-(4-Ethylthiazol-2-yl)-2-(4-(methylsulfonyl)phenyl)-3-(tetrahydro-2*H*-pyran-4-yl)-propanamide (9b)

Prepared by Method B as viscous white solid, yield 21.0% (purified by preparative TLC with EtOAc/1%MeOH/0.5%HOAc as the eluent); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (d, J = 8.4 Hz, 2H), 7.46 (d, J = 8.4 Hz, 2H), 6.55 (s, 1H), 3.90 (d, J = 11.7 Hz, 2H), 3.78 (t, J = 7.5 Hz, 1H), 3.26 (t, J = 11.4 Hz, 2H), 3.08 (s, 3H), 2.64 (m, 2H), 2.16 (m, 1H), 1.76 (m, 1H), 1.56 (t, J = 11.4 Hz, 2H), 1.27 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$+CD_3OD) δ 170.4, 157.9, 152.8, 145.2, 139.3, 128.9, 127.8, 106.8, 67.6, 48.8, 44.3, 40.3, 32.6, 32.4, 24.3, 12.9; EI-MS m/z 422 (M^+). Anal. Calcd for ($C_{20}H_{26}N_2O_4S_2$·HOAc·H_2O): C, 52.78; N, 5.60; H, 6.44. Found: C, 52.56; N, 5.45; H, 6.29.

5.1.2. *N*-(4-Isopropylthiazol-2-yl)-2-(4-(methylsulfonyl) phenyl)-3-(tetrahydro-2*H*-pyran-4-yl)-propanamide (9c)

White solid, yield 34.0%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.21 (br s, 1H), 7.90 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H), 6.56 (s, 1H), 3.93 (d, J = 11.7 Hz, 2H), 3.78 (t, J = 7.5 Hz, 1H), 3.28 (t, J = 11.4 Hz, 2H), 3.08 (s, 3H), 2.90 (m, 1H), 2.20 (m, 1H), 1.80 (m, 1H), 1.60 (t, J = 11.4 Hz, 2H), 1.31 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 157.9, 156.9, 144.9, 139.6, 128.9, 128.1, 106.0, 67.6, 49.4, 44.4, 40.4, 32.8, 32.6, 32.5, 30.6, 22.2, 22.1; EI-MS m/z 436 (M^+). Anal. Calcd for ($C_{21}H_{28}N_2O_4S_2$): C, 57.77, N 6.42; H, 6.46. Found: C, 8.06, N 5.95; H, 6.70.

5.1.3. *N*-(4-Isobutylthiazol-2-yl)-2-(4-(methylsulfonyl)phenyl)-3-(tetrahydro-2*H*-pyran-4-yl)-propanamide (9d)

White solid, yield 36.1%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.65 (br s, 1H), 7.86 (d, J = 8.1 Hz, 2H), 7.46 (d, J = 7.5 Hz, 2H), 6.53 (s, 1H), 3.89 (d, J = 10.8 Hz, 2H), 3.78 (t, J = 7.5 Hz, 1H), 3.27 (t, J = 11.7 Hz, 2H), 3.08 (s, 3H), 2.46 (d, J = 7.2 Hz, 2H), 2.17 (m, 1H), 1.91 (m, 1H), 1.78 (m, 1H), 1.57 (t, J = 10.5 Hz, 2H), 1.30 (m, 3H), 0.87 (d, J = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 157.0, 150.9, 144.9, 139.8, 128.8, 128.1, 108.8, 67.6, 49.4, 44.4, 40.6, 40.3, 32.9, 32.6, 32.5, 28.3, 22.32, 22.28; EI-MS m/z 450 (M^+). Anal. Calcd for ($C_{22}H_{30}N_2O_4S_2$): C, 58.64, N 6.22; H, 6.71. Found: C, 58.33, N 5.94; H, 6.84.

5.1.4. *N*-(4-*tert*-Butylthiazol-2-yl)-2-(4-(methylsulfonyl) phenyl)-3-(tetrahydro-2*H*-pyran-4-yl)-propanamide (9e)

White solid, yield 50.0%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.20 (br s, 1H), 7.90 (d, J = 8.1 Hz, 2H), 7.54 (d, J = 8.7 Hz, 2H), 6.54 (s, 1H), 3.92 (dd, J = 11.7, 2.7 Hz, 2H), 3.79 (t, J = 7.5 Hz, 1H), 3.29 (m, 2H), 3.09 (s, 3H), 2.19 (m, 1H), 1.77 (m, 1H), 1.61 (m, 2H), 1.30 (m, 3H), 1.21 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$+CD_3OD) δ 170.2, 160.6, 157.3, 145.2, 139.4, 128.9, 127.8, 105.2, 67.6, 49.0, 44.3, 40.1, 34.1, 32.6, 32.4, 29.6; EI-MS m/z 450 (M^+). Anal. Calcd for ($C_{22}H_{30}N_2O_4S_2$): C, 58.64; N, 6.22; H 6.71. Found: C, 58.33; N, 5.94; H 6.86.

5.1.5. *N*-(4-Cyclopropylthiazol-2-yl)-2-(4-(methylsulfonyl) phenyl)-3-(tetrahydro-2*H*-pyran-4-yl)propanamide (9f)

Red solid, yield 41.2%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.48 (br s, 1H), 7.87 (d, J = 8.1 Hz, 2H), 7.48 (d, J = 8.4 Hz, 2H), 6.50 (s, 1H), 3.90 (d, J = 11.1 Hz, 2H), 3.77 (t, J = 7.5 Hz, 1H), 3.27 (t, J = 11.4 Hz, 2H), 3.08 (s, 3H), 2.17 (m, 1H), 1.84 (m, 2H), 1.58 (t, J = 11.1 Hz, 2H), 1.30 (m, 3H), 0.86 (m, 2H), 0.72 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 157.1, 153.2, 144.9, 139.8, 128.8, 128.1, 105.9, 67.6, 49.4, 44.4, 40.3, 32.9, 32.6, 32.5, 12.0, 7.8, 7.7; EI-MS m/z 434 (M^+). HRMS Calcd for $C_{21}H_{26}N_2O_4S_2$: 434.1334. Found: 434.1323.

5.1.6. 2-(4-(Methylsulfonyl)phenyl)-*N*-(4-phenylthiazol-2-yl)-3-(tetrahydro-2*H*-pyran-4-yl)-propanamide (9g)

Yellow solid, yield 26.7%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.50 (br s, 1H), 7.86 (d, J = 6.9 Hz, 2H), 7.66 (d, J = 8.7 Hz, 2H), 7.43 (m, 3H), 7.27 (s, 1H), 6.94 (d, J = 8.7 Hz, 2H), 3.77 (d, J = 10.5 Hz, 2H), 3.30 (t, J = 7.5 Hz, 1H), 3.11 (m, 2H), 3.03 (s, 3H), 1.80 (m, 1H), 1.54 (m, 1H), 1.14 (m, 2H), 0.97 (m, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 170.4, 159.6, 149.4, 144.6, 139.5, 133.6, 129.3, 128.8, 128.2, 127.7, 125.9, 108.3, 67.5, 67.4, 49.0, 44.4, 40.2, 32.5, 32.2, 32.0; EI-MS m/z 470 (M^+). Anal. Calcd for ($C_{24}H_{26}N_2O_4S_2$·0.25H_2O): C, 60.67; N, 5.90; H 5.62. Found: C, 61.03; N, 5.48; H, 5.86.

5.1.7. *N*-(4-(4-Methoxyphenyl)thiazol-2-yl)-2-(4-(methylsulfonyl) phenyl)-3-(tetrahydro-2*H*-pyran-4-yl)propanamide (9h)

White solid, yield 55.0%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.93 (br s, 1H), 7.80 (d, J = 8.7 Hz, 2H), 7.60 (d, J = 8.1 Hz, 2H), 7.14 (s, 1H), 6.95 (d, J = 8.7 Hz, 2H), 6.84 (d, J = 8.1 Hz, 2H), 3.82 (s, 3H), 3.74 (d, J = 10.8 Hz, 2H), 3.21 (t, J = 7.2 Hz, 1H), 3.10 (t, J = 10.5 Hz, 2H), 3.03 (s, 3H), 1.81 (m, 1H), 1.45 (m, 1H), 1.13 (d, J = 11.7 Hz, 2H), 0.91 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 160.1, 159.7, 149.1, 144.7, 139.4, 128.1, 127.6, 127.3, 126.3, 114.6, 106.3, 67.5, 67.4, 55.3, 48.8, 44.3, 32.3, 32.2, 32.1; EI-MS m/z 500 (M^+). Anal. Calcd for ($C_{25}H_{28}N_2O_5S_2$·0.1H_2O): C, 59.76; N, 5.58; H, 5.66. Found: C, 59.57; N, 5.38; H, 5.67.

5.1.8. *N*-(4-(4-Fluorophenyl)thiazol-2-yl)-2-(4-(methylsulfonyl) phenyl)-3-(tetrahydro-2*H*-pyran-4-yl)propanamide (9i)

White solid, yield 51.2%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.19 (br s, 1H), 7.82 (m, 2H), 7.70 (d, J = 8.4 Hz, 2H), 7.19 (s, 1H), 7.11 (t, J = 8.7 Hz, 2H), 7.03 (d, J = 8.1 Hz, 2H), 3.80 (d, J = 10.5 Hz, 2H), 3.36 (t, J = 7.2 Hz, 1H), 3.15 (m, 2H), 3.05 (s, 3H), 1.93 (m, 1H), 1.54 (m, 1H), 1.24 (d, J = 12.0 Hz, 2H), 1.05 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 164.1, 161.6, 159.1, 148.6, 144.6, 139.7, 130.1, 128.3, 127.9, 127.8, 127.7, 116.2, 116.0, 107.8, 67.51, 67.48, 49.2, 44.4, 40.3, 32.6, 32.4, 32.3; EI-MS m/z 488 (M^+). Anal. Calcd for ($C_{24}H_{25}FN_2O_4S_2{\cdot}H_2O$): C, 56.90; N, 5.53; H, 5.37. Found: C, 56.71; N, 5.25; H, 5.11.

5.1.9. 2-(4-(Cyclopropylsulfonyl)phenyl)-*N*-(4-(prop-1-en-2-yl)thiazol-2-yl)-3-(tetrahydro-2*H*-pyran-4-yl)propanamide (9j)

White solid, 79.6% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.94 (br s, 1H), 7.70 (m, 2H), 7.12 (m, 2H), 6.88 (s, 1H), 5.77 (s, 1H), 5.16 (s, 1H), 3.86 (d, J = 10.1 Hz, 2H), 3.62 (t, J = 6.3 Hz, 1H), 3.23 (m, 2H), 2.46 (m, 1H), 2.12 (s, 3H), 1.98 (m, 1H), 1.67 (m, 1H), 1.46 (d, J = 10.5 Hz, 2H), 1.34 (m, 5H), 1.05 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 158.4, 150.4, 144.3, 139.8, 136.7, 128.4, 128.2, 114.2, 109.3, 67.61, 67.57, 49.2, 49.1, 40.2, 32.8, 32.7, 32.5, 32.4, 20.3, 6.2, 6.0; ESI-MS m/z 461 (M^+). Anal. Calcd for ($C_{23}H_{28}N_2O_4S_2$): C, 59.97; N, 6.08; H, 6.13. Found: C, 59.87; N, 6.02; H, 6.21.

5.1.10. (R)-2-(4-(Cyclopropylsulfonyl)phenyl)-*N*-(4-isopropyl thiazol-2-yl)-3-(tetrahydro-2*H*-pyran-4-yl)propanamide (R-9k)

White foam, yield 61.2%, 95% ee; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.77 (br s, 1H), 7.82 (d, J = 7.8 Hz, 2H), 7.43 (d, J = 8.4 Hz, 2H), 6.51 (s, 1H), 3.88 (d, J = 11.4 Hz, 2H), 3.80 (t, J = 7.5 Hz, 1H), 3.26 (t, J = 11.1 Hz, 2H), 2.88 (m, 1H), 2.53 (m, 1H), 2.17 (m, 1H), 1.72 (m, 1H), 1.57 (d, J = 11.4 Hz, 2H), 1.33 (m, 5H), 1.20 (d, J = 6.9 Hz, 6H), 1.02 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.8, 157.8, 157.0, 144.7, 139.5, 128.6, 128.1, 105.8, 67.5, 49.1, 40.4, 32.74, 32.68, 32.5, 32.4, 30.6, 22.1, 22.0, 6.0, 5.9; EI-MS m/z 462 (M^+). Anal. Calcd for ($C_{23}H_{30}N_2O_4S_2$): C, 59.71; N, 6.06; H, 6.54. Found: C, 59.58; N, 5.77; H, 6.66.

5.1.11. *N*-(4,5-Dimethylthiazol-2-yl)-2-(4-(methylsulfonyl) phenyl)-3-(tetrahydro-2*H*-pyran-4-yl)propanamide (9l)

Viscous white solid, yield 30.0%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (d, J = 8.4 Hz, 2H), 7.46 (d, J = 8.4 Hz, 2H), 3.90 (m, 3H), 3.26 (t, J = 11.4 Hz, 2H), 2.29 (s, 3H), 2.21 (s, 3H), 2.16 (m, 1H), 1.79 (m, 1H), 1.58 (t, J = 11.4 Hz, 2H), 1.29 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$+CD_3OD) δ 170.7, 155.8, 144.6, 139.4, 137.6, 128.9, 127.8, 120.5, 67.5, 48.7, 46.8, 44.1, 40.1, 32.6, 32.4, 32.3, 12.7, 10.5; EI-MS m/z 422 (M^+). This compound was converted to its HBr salt for analysis. Anal. Calcd for ($C_{20}H_{26}N_2O_4S_2{\cdot}1.5HBr{\cdot}1.5H_2O$): C, 42.07; N, 4.91; H, 5.38. Found: C, 42.37; N, 4.80; H, 5.55.

5.1.12. *N*-(5-Isopropyl-4-methylthiazol-2-yl)-2-(4-(methyl sulfonyl)phenyl)-3-(tetrahydro-2*H*-pyran-4-yl)propanamide (9m)

White solid, yield 42.0% (purified by preparative TLC with EtOAc/1%MeOH/0.5%HOAc as the eluent); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (dd, J = 8.4, 2.1 Hz, 2H), 7.38 (dd, J = 8.4, 2.7 Hz, 2H), 3.89 (d, J = 8.4 Hz, 2H), 3.69 (t, J = 7.5 Hz, 2H), 3.25 (t, J = 11.1 Hz, 2H), 3.15 (m, 1H), 3.06 (s, 3H), 2.22 (s, 3H), 2.15 (m, 1H), 1.71 (m, 1H), 1.54 (t, J = 10.2 Hz, 2H), 1.31 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5, 154.1, 144.9, 139.7, 139.4, 134.5, 128.7, 128.0, 67.6, 49.4, 44.4, 40.3, 32.8, 32.6, 27.0, 24.7, 14.7; EI-MS m/z 450 (M^+). Anal. Calcd for ($C_{22}H_{30}N_2O_4S_2{\cdot}0.6HOAc$): C, 57.26; N, 5.76; H, 6.71. Found: C, 57.53; N, 5.39; H, 7.00.

5.1.13. *N*-(5-Bromo-4-isopropylthiazol-2-yl)-2-(4-(cyclopropyl sulfonyl)phenyl)-3-(tetrahydro-2*H*-pyran-4-yl)propanamide (9n)

Yellow foam, 46.9%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.75 (br s, 1H), 7.81 (d, J = 8.1 Hz, 2H), 7.47 (d, J = 8.7 Hz, 2H), 3.91 (dd, J = 11.7, 3.0 Hz, 2H), 3.84 (t, J = 7.8 Hz, 1H), 3.29 (t, J = 10.8 Hz, 2H), 3.13 (m, 1H), 2.56 (m, 1H), 2.19 (m, 1H), 1.71 (m, 1H), 1.59 (d, J = 11.1 Hz, 2H), 1.37 (m, 5H), 1.14 (d, J = 6.6 Hz, 3H), 1.11 (d, J = 7.2 Hz, 3H), 1.04 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.9, 156.2, 154.4, 144.6, 139.5, 128.8, 128.2, 97.3, 67.6, 49.0, 40.6, 32.8, 32.7, 32.6, 32.5, 28.1, 21.6, 6.13, 6.08; EI-MS m/z 542 (M^+). HRMS Calcd for $C_{23}H_{29}BrN_2O_4S_2$: 542.0752. Found: 542.0793.

5.1.14. 2-(4-(Cyclopropylsulfonyl)phenyl)-*N*-(5-iodo-4-isopropylhiazol-2-yl)-3-(tetrahydro-2*H*-pyran-4-yl)propanamide (9o)

Yellow solid, 62% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.55 (s, 1H), 7.82 (d, J = 8.4 Hz, 2H), 7.46 (d, J = 7.8 Hz, 2H), 3.91 (dd, J = 12.0, 2.7 Hz, 2H), 3.82 (t, J = 7.2 Hz, 1H), 3.29 (t, J = 11.4 Hz, 2H), 3.08 (m, 1H), 2.56 (m, 1H), 2.19 (m, 1H), 1.68 (m, 1H), 1.59 (d, J = 11.4 Hz, 2H), 1.38 (m, 5H), 1.11 (m, 6H), 1.04 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.9, 160.9, 159.4, 144.5, 139.7, 128.7, 128.2, 67.6, 62.0, 49.1, 40.6, 32.9, 32.8, 32.7, 32.6, 30.2, 21.8, 6.1; EI-MS m/z 588 (M^+). Anal. Calcd for ($C_{23}H_{29}IN_2O_4S_2$): C, 46.94; N, 4.76; H, 4.97. Found: C, 46.77; N, 4.58; H, 4.94.

5.1.15. *N*-(6-Fluorobenzo[*d*]thiazol-2-yl)-2-(4-(methylsulfonyl) phenyl)-3-(tetrahydro-2*H*-pyran-4-yl)propanamide (9p)

White solid, yield 36.0%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (d, J = 8.1 Hz, 2H), 7.66 (m, 1H), 7.53 (m, 1H), 7.38 (d, J = 8.4 Hz, 2H), 7.18 (m, 1H), 3.90 (d, J = 11.1 Hz, 2H), 3.80 (t, J = 7.5 Hz, 2H), 3.26 (t, J = 11.1 Hz, 2H), 3.09 (s, 3H), 2.17 (m, 1H), 1.77 (m, 1H), 1.54 (d, J = 11.7 Hz, 2H), 1.31 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 160.9, 158.5, 157.9, 144.5, 144.4, 139.8, 133.0, 128.8, 128.1, 121.5, 115.0, 114.9, 109.1, 109.0, 67.56, 67.52, 49.4, 44.4, 40.3, 32.8, 32.5; EI-MS m/z 462 (M^+). Anal. Calcd for ($C_{22}H_{23}FN_2O_4S_2$): C, 57.12; N, 6.06; H, 5.01. Found: C, 57.02; N, 5.66; H, 5.30.

5.1.16. 2-(4-(Cyclopropylsulfonyl)phenyl)-3-(tetrahydro-2*H*-pyran-4-yl)-*N*-(4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-yl) propanamide (9q)

White foam, yield 78.6%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (d, J = 8.1 Hz, 2H), 7.39 (d, J = 8.4 Hz, 2H), 3.87 (d, J = 11.4 Hz, 2H), 3.74 (t, J = 7.5 Hz, 1H), 3.24 (t, J = 11.4 Hz, 2H), 2.68 (s, 2H), 2.60 (s, 2H), 2.48 (m, 1H), 2.13 (m, 1H), 1.83 (m, 4H), 1.73 (m, 1H), 1.54 (t, J = 10.2 Hz, 2H), 1.32 (m, 5H), 1.04 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 155.7, 144.5, 143.6, 139.8, 128.6, 128.1, 123.3, 67.6, 49.2, 40.1, 32.8, 32.5, 32.4, 26.2, 23.1, 22.8, 22.7, 6.1, 6.0; EI-MS m/z 474 (M^+). Anal. Calcd for ($C_{24}H_{30}N_2O_4S_2$): C, 60.73; N, 5.90; H, 6.37. Found: C, 60.37; N, 5.68; H, 6.36.

5.1.17. 2-(4-(Cyclopropylsulfonyl)phenyl)-*N*-(5,6-dihydro-4*H*-cyclopenta[*d*]thiazol-2-yl)-3-(tetrahydro-2*H*-pyran-4-yl) propanamide (9r).

White foam, yield 36.9%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (d, J = 8.4 Hz, 2H), 7.39 (d, J = 8.4 Hz, 2H), 3.87 (d, J = 11.7 Hz, 2H), 3.77 (t, J = 7.5 Hz, 1H), 3.23 (t, J = 11.7 Hz, 2H), 2.89 (m, 2H), 2.74 (m, 2H), 2.46 (m, 3H), 2.15 (m, 1H), 1.73 (m, 1H), 1.54 (t, J = 11.4 Hz, 2H), 1.31 (m, 5H), 1.03 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.8, 161.5, 154.6, 144.4, 139.8, 129.1, 128.5, 128.2, 67.6, 49.2, 40.2, 32.8, 32.6, 27.6, 27.3, 26.6, 6.1, 6.0; EI-MS m/z 460 (M^+). Anal. Calcd for ($C_{23}H_{28}N_2O_4S_2$): C, 59.97; N, 6.08; H, 6.13. Found: C, 59.67; N, 6.90; H, 6.38.

5.1.18. 2-(4-(Cyclopropylsulfonyl)phenyl)-*N*-(5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*] pyridin-2-yl)-3-(tetrahydro-2*H*-pyran-4-yl)propanamide (9s)

Yellow solid, yield 65.3%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, J = 8.7 Hz, 2H), 7.40 (d, J = 8.7 Hz, 2H), 3.88 (d, J = 11.4 Hz, 2H), 3.75 (t, J = 7.5 Hz, 1H), 3.59 (s, 2H), 3.24 (t, J = 10.8 Hz, 2H), 2.74 (m, 4H), 2.49 (m, 4H), 2.14 (m, 1H), 1.74 (m, 1H), 1.54 (t, J = 12.6 Hz, 2H), 1.31 (m, 5H), 1.03 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 155.8, 144.3, 142.5, 139.8, 128.6, 128.2, 120.8, 67.6, 52.4, 51.9, 49.3, 45.2, 40.2, 32.8, 32.5, 32.4, 26.6, 6.1; EI-MS m/z 489 (M^+). Anal. Calcd for ($C_{24}H_{31}N_3O_4S_2\cdot H_2O$): C, 56.78; N, 8.28; H, 6.55. Found: C, 56.81; N, 8.50; H, 6.20.

5.1.19. 2-(4-(Cyclopropylsulfonyl)phenyl)-*N*-(4-oxo-4,5-dihydrothiazol-2-yl)-3-(tetrahydro-2*H*-pyran-4-yl)propanamide (9t)

Yellow solid, yield 35.4%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (d, J = 8.4 Hz, 2H), 7.57 (d, J = 8.4 Hz, 2H), 4.82 (t, J = 7.2 Hz, 1H), 3.91 (m, 2H), 3.80 (d, J = 3.0 Hz, 2H), 3.26 (t, J = 11.7 Hz, 2H), 2.43 (m, 1H), 2.13 (m, 1H), 1.77 (m, 1H), 1.70 (d, J = 13.2 Hz, 1H), 1.58 (d, J = 13.2 Hz, 1H), 1.33 (m, 5H), 1.02 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 186.8, 185.1, 175.5, 144.0, 140.0, 128.9, 128.2, 67.7, 48.4, 41.0, 36.3, 32.9, 32.7, 32.6, 32.5, 6.0; MALDI-MS m/z (%) 437.0 (MH^+, 46%), 459.0 (M^++Na, 100%). HRMS Calcd for ($C_{20}H_{24}N_2NaO_5S_2$): 459.10234. Found: 459.10282.

5.1.20. (Z)-*N*-(5-Benzylidene-4-oxo-4,5-dihydrothiazol-2-yl)-2-(4-(cyclopropylsulfonyl)phenyl)-3-(tetrahydro-2*H*-pyran-4-yl) propanamide (9u)

Brown solid, yield 63%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.85 (s, 1H), 8.02 (s, 1H), 7.83 (d, J = 8.4 Hz, 2H), 7.66 (d, J = 8.1 Hz, 4H), 7.51 (m, 3H), 5.16 (t, J = 7.2 Hz, 1H), 3.92 (t, J = 5.7 Hz, 2H), 3.32 (m, 2H), 2.42 (m, 1H), 2.18 (m, 1H), 1.83 (m, 1H), 1.76 (d, J = 11.7 Hz, 1H), 1.67 (d, J = 12.3 Hz, 1H), 1.32 (m, 5H), 1.00 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.8, 177.3, 175.8, 144.2, 139.8, 138.8, 133.1, 131.2, 130.8, 129.3, 128.9, 128.1, 124.3, 67.7, 48.3, 41.2, 32.83, 32.78, 32.7, 5.9; ESI-MS m/z 523.0 (M^+). Anal. Calcd for ($C_{27}H_{28}N_2O_5S_2\cdot 0.1H_2O$): C, 61.60; N, 5.32; H, 5.40. Found: C, 61.30; N, 5.68; H, 5.43.

5.1.21. (Z)-2-(4-(Cyclopropylsulfonyl)phenyl)-*N*-(5-(2-methyl propylidene)-4-oxo-4,5-dihydro thiazol-2-yl)-3-(tetrahydro-2*H*-pyran-4-yl)propanamide (9v)

Yellow solid, yield 36.0%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, J = 8.4 Hz, 2H), 7.58 (d, J = 8.4 Hz, 2H), 7.06 (d, J = 9.9 Hz, 1H), 4.96 (t, J = 7.2 Hz, 1H), 3.89 (m, 2H), 3.27 (m, 2H), 2.61 (m, 1H), 2.41 (m, 1H), 2.12 (m, 1H), 1.76 (m, 1H), 1.70 (d, J = 13.2 Hz, 1H), 1.60 (d, J = 12.3 Hz, 1H), 1.30 (m, 5H), 1.19 (t, J = 5.7 Hz, 6H), 1.00 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.2, 176.6, 176.0, 149.4, 144.3, 139.8, 128.9, 128.1, 126.3, 67.7, 48.5, 41.0, 32.8, 32.73, 32.70, 32.6, 21.5, 6.0; ESI-MS m/z 489 (M^+). Anal. Calcd for ($C_{24}H_{30}N_2O_5S_2$): C, 58.75; N, 5.71; H, 6.16. Found: C, 59.09; N, 5.91; H, 6.35.

5.2. Biological assay

5.2.1. Preparation of recombinant glucokinase protein

cDNA of human glucokinase (MGC: 1742, purchased from OriGene Technologies, USA) was subcloned into the pET28a (+) expression vector, and expressed in *Escherichia coli* strain BL21 (DE3). The NH_2 terminal end of $(His)_6$-tag glucokinase fusion protein was purified by Ni-NTA metal chelate affinity chromatography and stored at −80 °C in 50 mM Tris–HCl pH 7.4, 1 mM dithiothreitol (DTT), 50 mM NaCl, and 10% glycerol.[42]

5.2.2. Glucokinase enzymatic assay

The GK activity was assessed spectrometrically by a coupled reaction with glucose-6-phosphate dehydrogenase (G6PDH).[21,42,43] Briefly, GK catalyzes glucose phosphorylation to generate glucose-6-P, which was oxidized by the G6PDH with the concomitant reduction of NADPH. The product NADPH was then monitored by the increase rate of absorbance at 340 nm in a plate reader (Spectra-Max 190; Molecular Devices, USA). All compounds were prepared in DMSO. The assay was performed in 96-well plates in a final volume of 100 μL containing 50 mM HEPES pH 7.4, 5 mM glucose, 25 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 1 mM ATP, 1 mM NADP, 2.5 U/mL G6PDH, 0.5 μg $(His)_6$-glucokinase, and test compounds. The velocities of the enzyme reaction were expressed as mOD/min, and the fold activation of the enzyme was achieved by comparing with control (GK activation with only DMSO was considered as 100%). For EC_{50} determination, six different concentrations of compounds were tested in the assay, and the fold changes in activity versus controls were fitted to sigmoidal curve using a four parameter logistic model in GraphPad Prism 4.

5.2.3. Isolation and culture of rat primary hepatocytes

Overnight fasted male Sprague–Dawley rats (180–250 g) were used. Hepatocytes were isolated by a two-step in situ liver perfusion with collagenase as described in the literature.[43,44] The viability of isolated hepatocytes were over 85% excluded by Tapan blue (Sigma). Cells were suspended in MEM (Invitrogen) supplemented with 10% fetal bovine serum (v/v), 100 U/mL penicillin, 100 μg/mL streptomycin, 10 nM insulin, and 10 nM dexamethasone. For experiments, 3.5×10^5 cells/mL cells were planted onto gelatin (0.5%, w/v) coated 24-well-plate (Corning), or 7–8 $\times$ 10^5 Cells onto 6-well-plate. After 4 h incubation (37 °C; 5% CO_2/95% air v/v) to allow cell attachment, the medium was then replaced with the same but without serum for another 16–20 h further culture. Cell protein was determined by the Bio-Rad protein assay kit.

5.2.4. Glucose metabolism in rat primary hepatocytes

2-Deoxy-D-[^{3}H]-glucose uptake was performed in cultured hepatocytes. Cells were washed and incubated in serum free MEM containing 5.5 mM glucose, 4 μCi/mL 2-deoxy-D-[3H]-glucose with or without test compounds for 4 h. The reaction was terminated and washed three times with ice-cold phosphate buffered saline (PBS), and then cells were lysed with 0.5 M NaOH. Portions of cell lysates were used for scintillation counting, and the results were expression by increased percentage versus control.

In glycogen accumulation assay, cells were incubated in MEM containing 5.5 mM glucose with or without test compounds for time and course response. Cells were washed with ice-cold 0.9% NaCl to terminate the reaction and then lysed by ultrasound. The cell lysates were treated with amyloglucosidase (exo-1,4-α-D-glucosidase) for 2 h at 40 0C,PH 4.8 as described in the literature.[44–46] The total free glucose were determined by a glucose assay kit (Shen Tong bio-company, Nanjing, PR China), with subtracted by the cellular free glucose, the glycogen content levels were expressed as increased percentage versus control.

5.2.5. Animal studies

Male ICR mice (8 weeks old) and diabetic female C57Bl/6J *ob/ob* mice (7–8 weeks), housed with free access to diet and reversed light cycles, were used. During the studies, food was deprived for 5 h in ICR mice, or 3 h in *ob/ob* mice, while free access to water was provided. The ICR mice were assigned to different groups based on the body weight, whereas the *ob/ob* mice were assigned based on the 3-h fasting blood glucose values (first criterion) and body weight (second criterion). Mice were then orally dosed with test compounds or vehicle (0.5% carboxymethylcellulose). Blood glucose levels were measured via blood sample from tail vein by

using an ACCU-CHEK® Advantage (Roche) at the indicated time after dosing.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.04.038.

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